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QUANTITATIVE ANALYSIS OF CERTAIN WING AND GENITALIA CHARACTERS OF PIERIS IN WESTERN NORTH AMERICA

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ALL SPECIES OF *Pieris* which occur in western North America were named in the nineteenth century at which time wing coloration and pattern were the most obvious characteristics used for identification. Great variations are present between various species of the genus *Pieris* with respect to these characters. Such variations have contributed to disagreement concerning the number of species which occur in North America. Some authors list seven species: *P. rapae, P. napi, P. virginiensis, P. sisymbrii, P. beckeri, P. protodice* and *P. occidentalis* (Klots 1932, McDunnough 1939). Abbott et al (1960) in a statistical study utilized six morphological characters which included the length and the width of the front wing, the length of the hind wing, body length and the degrees of melanization of the upper and lower sides of the wings of the specimens. They found that there is a continuous variation in wing sizes, color and marking between *P. protodice, P. occidentalis* and *P. sisymbrii.* Such variations were found associated with geographical location and climate. They suggested that *P. protodice, P. occidentalis* and *P. sisymbrii* were a single species. Klots (1961) in his latest *Pieris* list, cited only six species, excluding *P. occidentalis.* Hovanitz (1962) combined the distribution of *P. occidentalis* and *P. protodice* in a geographical study because of uncertainty of how to separate them. Evidence for considering them as separate species is given in this paper. Hovanitz (1963) also indicated that *P. napi* and *P. virginiensis* ought to be considered as a single species at the present time for nomenclatural purposes pending more complete evidence of their possible sympatric distribution. Since *P. virginiensis* occurs only in the region east of the Rocky Mountains in North America, it is excluded from consideration in this paper. In the present paper, mainly morphological characters are studied, which are intended to aid in the elucidation of the biological relationships between these various species of *Pieris.*

[1]*This work was supplementary to work aided by a grant from the National Science Foundation.*

MATERIALS AND METHODS

Five hundred specimens have been studied from the collections gathered by William Hovanitz and the author throughout the region in recent years. Despite this fact, this study is not intended of an exhaustive geographical study.

The wing characters used in this study include: (1) wing color and pattern, of which the detailed description will not be given; however, the most important characters are outlined in the text, and (2) wing venation, which is relatively uniform in the genus *Pieris*. Differences have been found in the length of the R_3, and in the location of R_2 in respect of M_2 and (3) the androconial scales which are the scent scales found with the black scales in the dot on or near the discal vein on the male fore wings.

The characters used for the male genitalia include (1) the uncus, (2) the juxta, (3) the saccus and (4) the penis. For the female genitalia, only the signum bursa has been used; this is a chitinized structure on the corpus bursa.

The overall size of the insects influences the size of their internal structures. To make this size difference of negligible influence, the simple ratio: the length of the left front wing against the length of a particular structure has been used for aiding in the elimination of such possible differences.

$$\text{Ratio} = \frac{\text{the length of a given structure}}{\text{the length of left front wing}} \text{ X } 100$$

The terminology of structural parts employed here has been adapted from Klots (1931). The color terms whenever used in the text follow the "Dictionary of Color" by Maerz and Paul (1930).

I. *P. rapae* and *P. napi*

Color and pattern (fig. 1a): *P. rapae* and *P. napi* are different from other species by lacking a dot on the discal vein of the fore wings. Most samples of *P. rapae* and *P. napi* are easily separated, except the all-white forms of both species.

Venation: The mean ratio (table 1) between the length of the left fore wing and the length of the discal cell are statistically different for these two species. The probability that the difference might be due to chance alone is less than 0.001 when the degree of freedom is 45 (as tested by "*t*"). The histogram (fig. 3) shows however that the range of ratios is continuous from one species to another. In actual fact, however, no overlap has been observed in the investigation between these two species though it may occur when the sample size is larger.

Location of the discal cell, and method of measuring venation are illustrated in fig. 2.

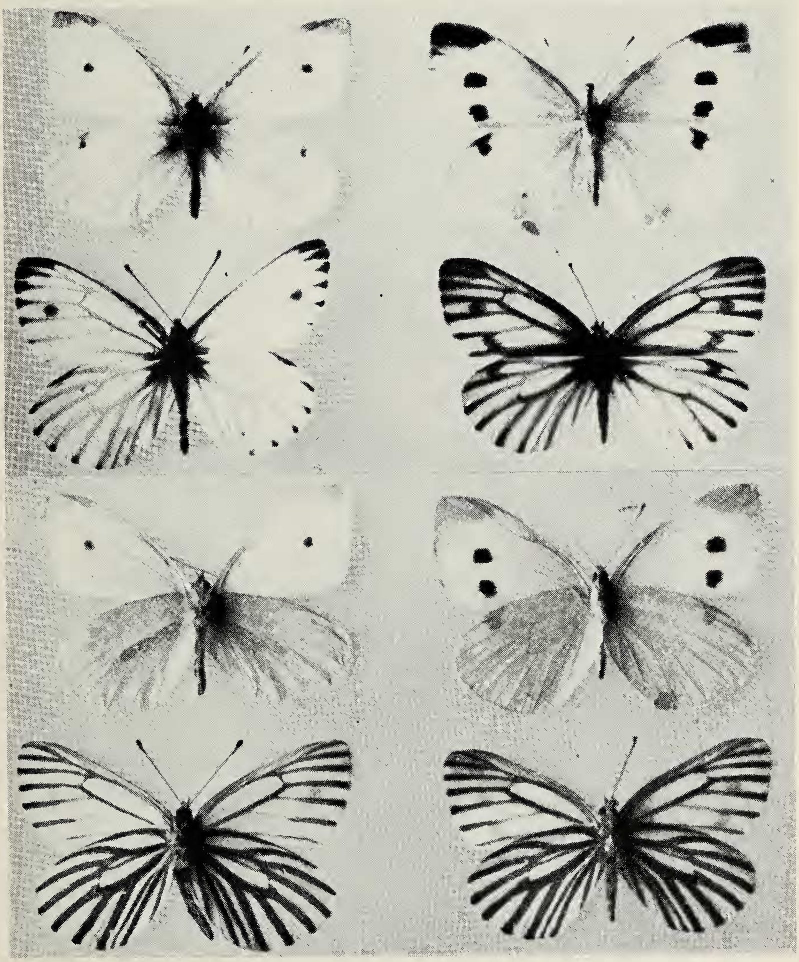

Fig. 1a. *Pieris rapae* and *Pieris napi.* Male on left, female on right. Upper four are upper side;lower four are lower side.

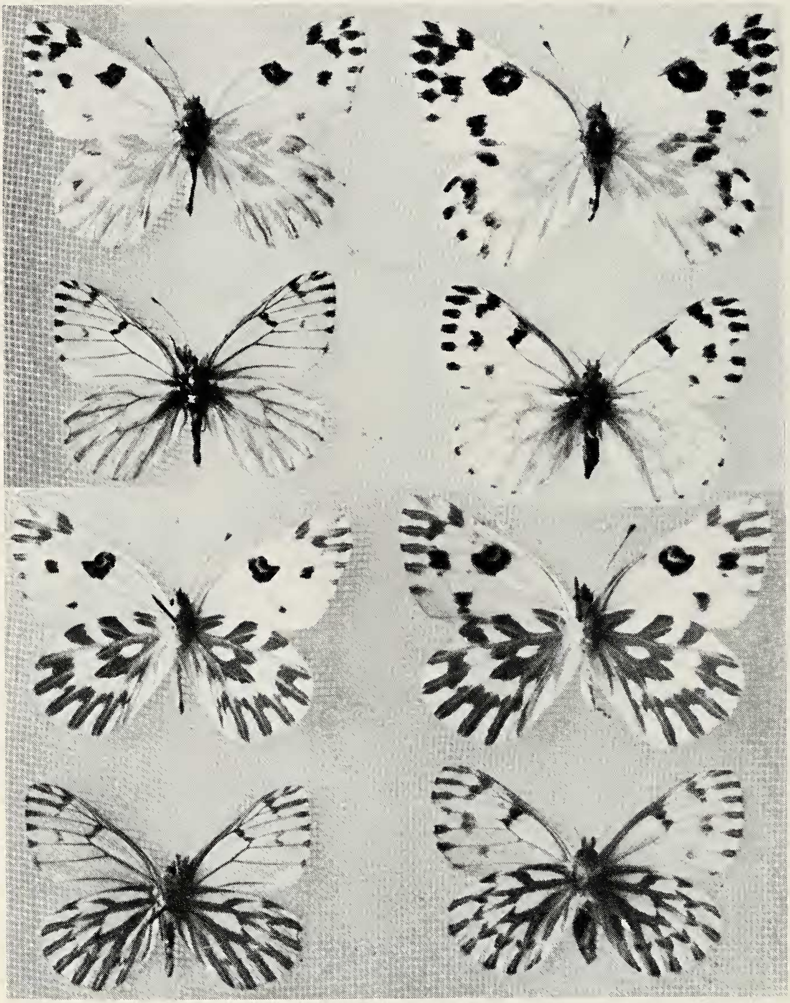

Fig. 1b. *Pieris beckeri* and *Pieris sisymbrii.* Male on left, female on right. Upper four are upper side, lower four are lower side.

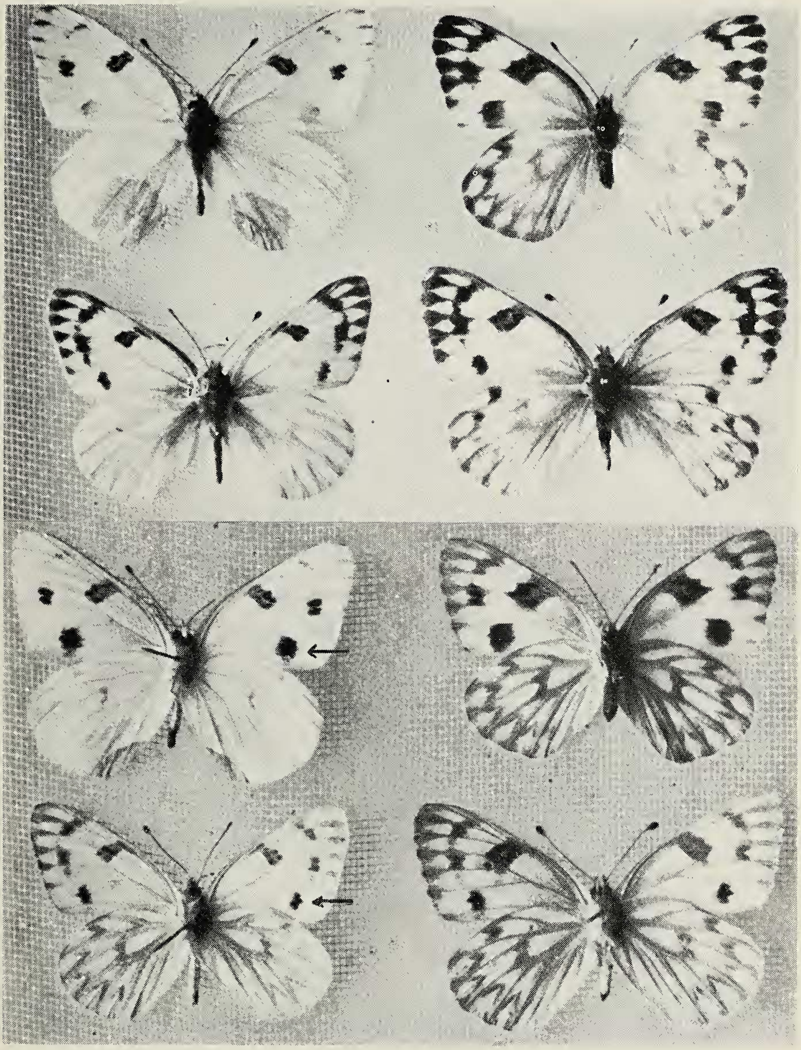

Fig. 1c. *Pieris protodice* and *Pieris occidentalis.* Male on left, female on right. Upper four are upper side, lower four are lower side.

Table 1 Ratios between length of discal cell and length of front wing

Species	No.	Mean length front wing	Mean length discal cell	Ratio	S. D.	S. E.	t	Probability
P. rapae	25	23.18 mm	12.44 mm	53.66	1.85	0.37	12.24	0.001
P. napi	20	21.79 mm	13.10 mm	60.15	1.65	0.37		

Table 2 Ratios between width of androconia and length of front wing

Species	No.	Mean length front wing	Mean width androconial stalk	Ratio	S. D.	S. E.	t	Probability
P. rapae	20	23.43 mm	0.12 mm	0.50	0.07	0.016	19.39	0.001
P. napi	20	23.55 mm	0.27 mm	1.14	0.13	0.029		

S. D. = Standard deviation; S. E. = Standard error

Androconia (fig. 4, 5, table 2): Both *P. rapae* and *P. napi* have androconial scales with broadened tops and two lobes. A difference is found on the width of the scale's stalk which for *P. rapae* is narrower than *P. napi*. The probability as shown by the "*t*" test that the difference between the mean ratio of these two species is due to chance alone is less than 0.001 for a degree of freedom of 40. The histogram (fig. 5) shows that there is a wide gap between the ratios. This is one of the characters that can be used to separate the all white forms of a *P. rapae* and *P. napi*.

Male genitalia: (1) the uncus (figs. 6, 7, table 3) is more slender and less curved at the ventral side for *P. rapae* and *P. napi* than for the other four species in the genus *Pieris*. The ratios: the length of the uncus against the length of the fore wing range from 2.07 to 2.84 for *P. rapae*, and from 2.69 to 3.05 for P. *napi*. The probability that this difference might be due to chance alone is 0.025 for a degrees of freedom of 18 as tested by *t:*

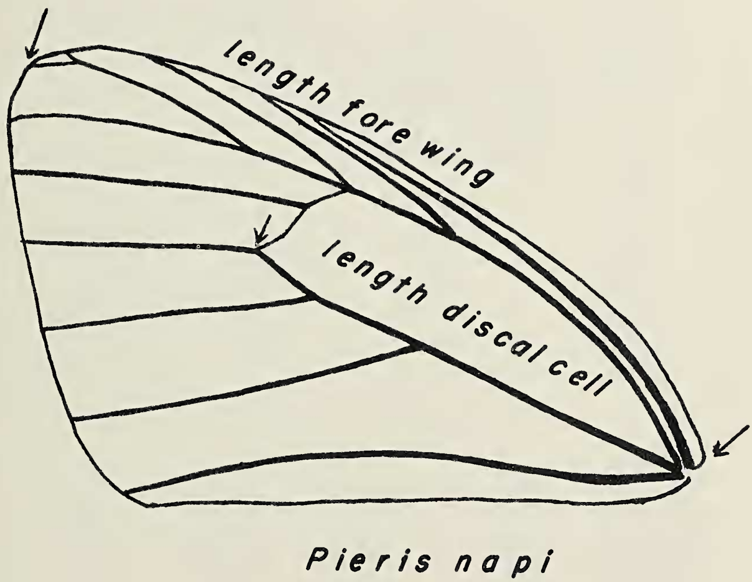

Fig. 2. Location of discal cell and points of measurement

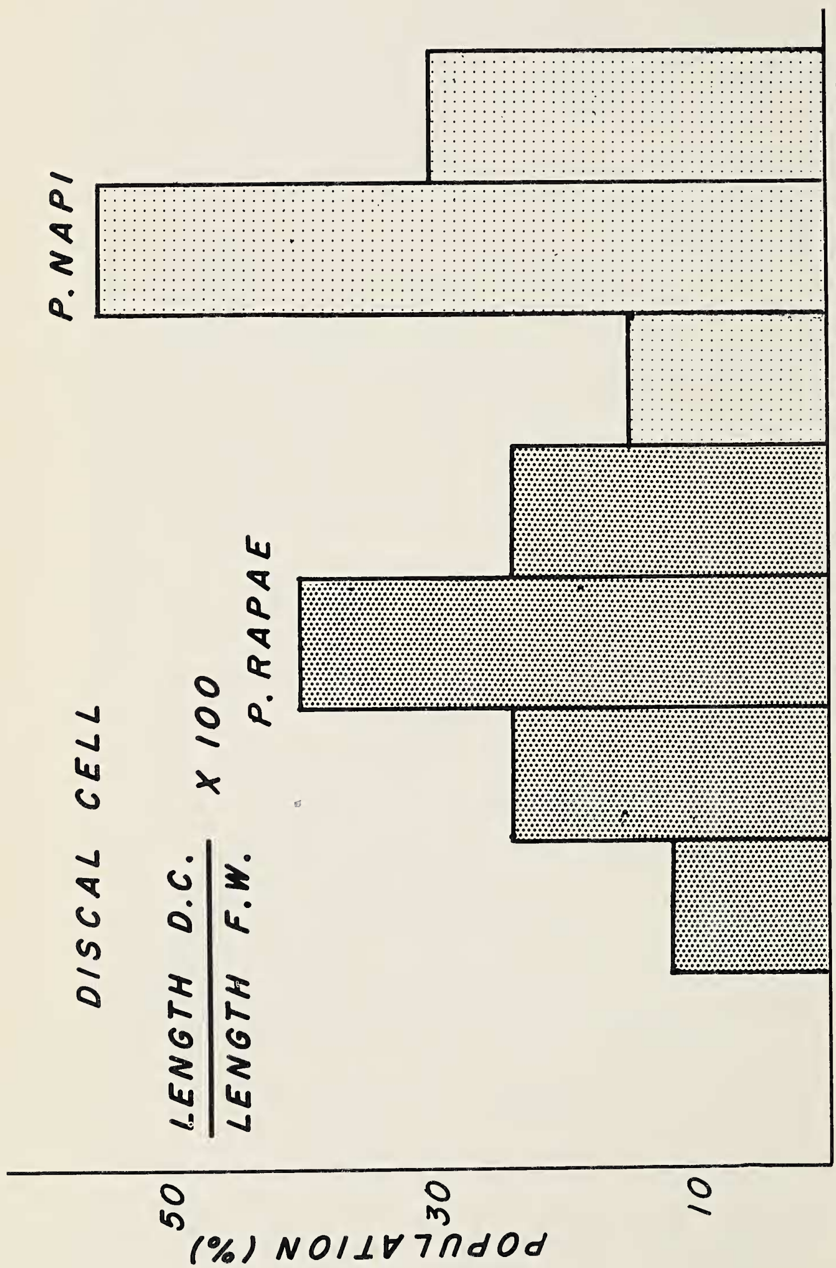

Fig. 3. Histogram illustrating the differences in ratios distribution between *P. rapae* and *P. napi*

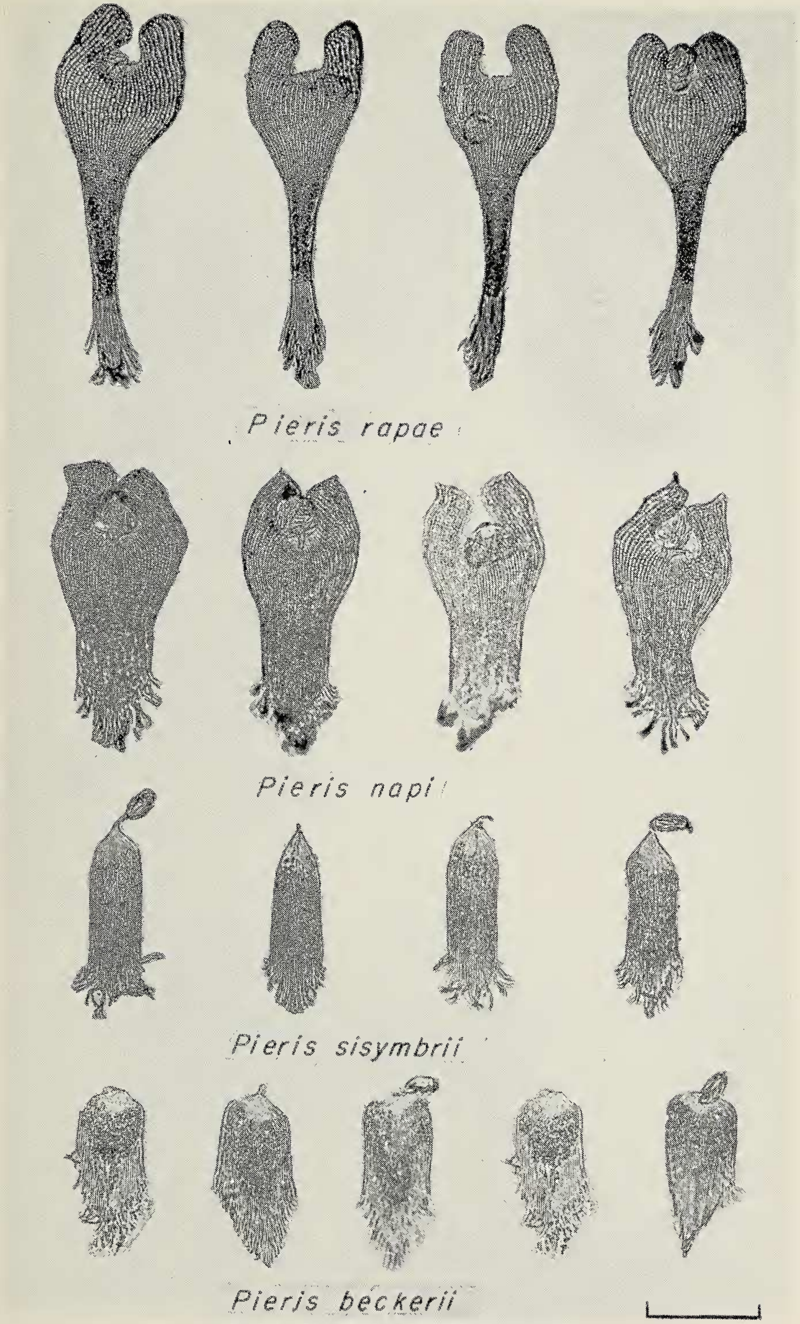

Fig. 4. Androconial scales (400x)

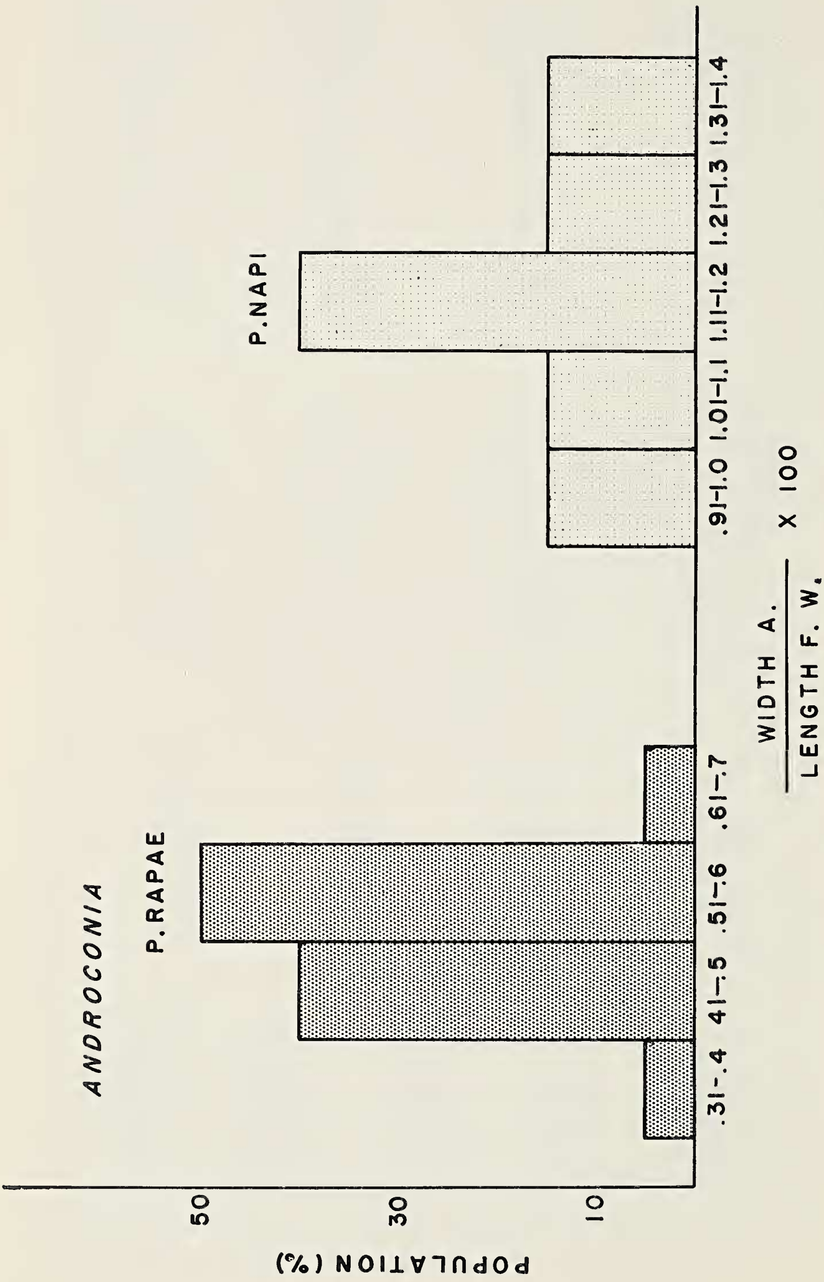

Fig. 5. Histogram illustrating the differences in ratios distribution between *P. rapae* and *P. napi*

Table 3 Ratios between length of various genital structures and length of front wing

Uncus

Species	No.	Mean length of uncus	Mean length of front wing	Mean of ratio	S. D.	S. E.	t	Probability
rapae	10	0.59 mm	22.6 mm	2.61	0.18	0.05	2.5	0.025
napi	8	0.70 mm	24.8 mm	2.81	0.12	0.04		
sisymbrii	11	0.55 mm	20.34 mm	2.71	0.23	0.07	3.54	0.005
beckeri	9	0.54 mm	24.18 mm	2.25	0.33	0.11		
protodice	37	0.48 mm	22.5 mm	2.12	0.22	0.04	1.38	0.2 +
occidentalis	25	0.56 mm	23.4 mm	2.40	0.22	0.04		

(2) The juxta (fig. 7) is quite different in shape between *P. rapae* and *P. napi*. The lower end of the juxta of *P. rapae* is triangular; however, in *P. napi,* this structure is reduced in size and not hollowed out.

(3) The saccus and penis (fig 8, table 4) show no significant differences between *P. rapae* and *P. napi*.

Signum bursa (fig. 8): This structure is again a good character to separate *P. rapae* and *P. napi*. The signum bursa of *P. rapae* is like a pair of kidneys; the central part is less toothed. In *P. napi,* it has a long unchitinized tail which is so distinctive that there is no problem in separating this species from others in the genus *Pieris*.

The samples of *P. rapae* came from *(1)* Arcadia, Los Angeles County, California (5 individuals), *(2)* Newport Beach, Orange Co., Calif. (5), *(3)* Riverside, Riverside Co. Calif. (5), (4) Bishop, Inyo Co. Calif. (5), *(5)* Klamath Falls, Klamath Co. Oregon (5), and (6) Satus Creek, Yakima Co. Washington (5). The samples of *P. napi* came from *(1)* Lopez Canyon, San Luis Obispo Co. Calif. (5), (2) Berkeley, Calif. (1), (3) Utah (1), (4) Hurricane Ridge, Olympic National Park, Wash. (10), (6) British Columbia (2), (7) Yukon Territory (6), and (8) Alaska (3).

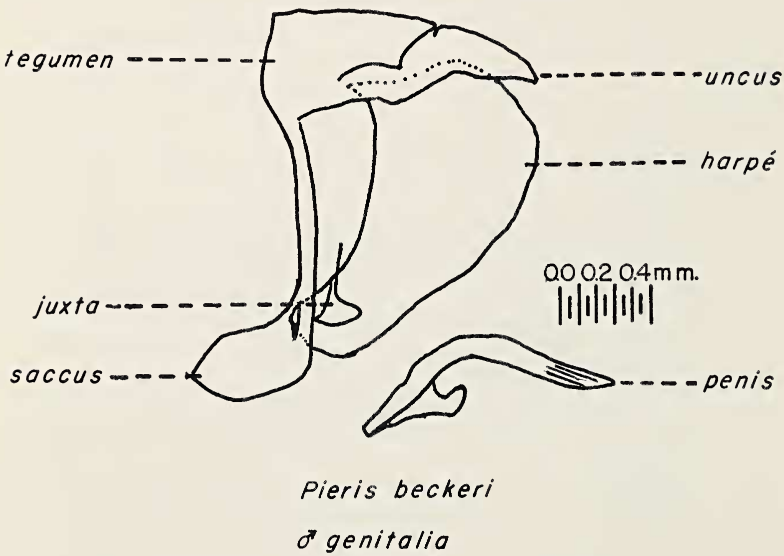

Fig. 6. The lateral view of male genitalia

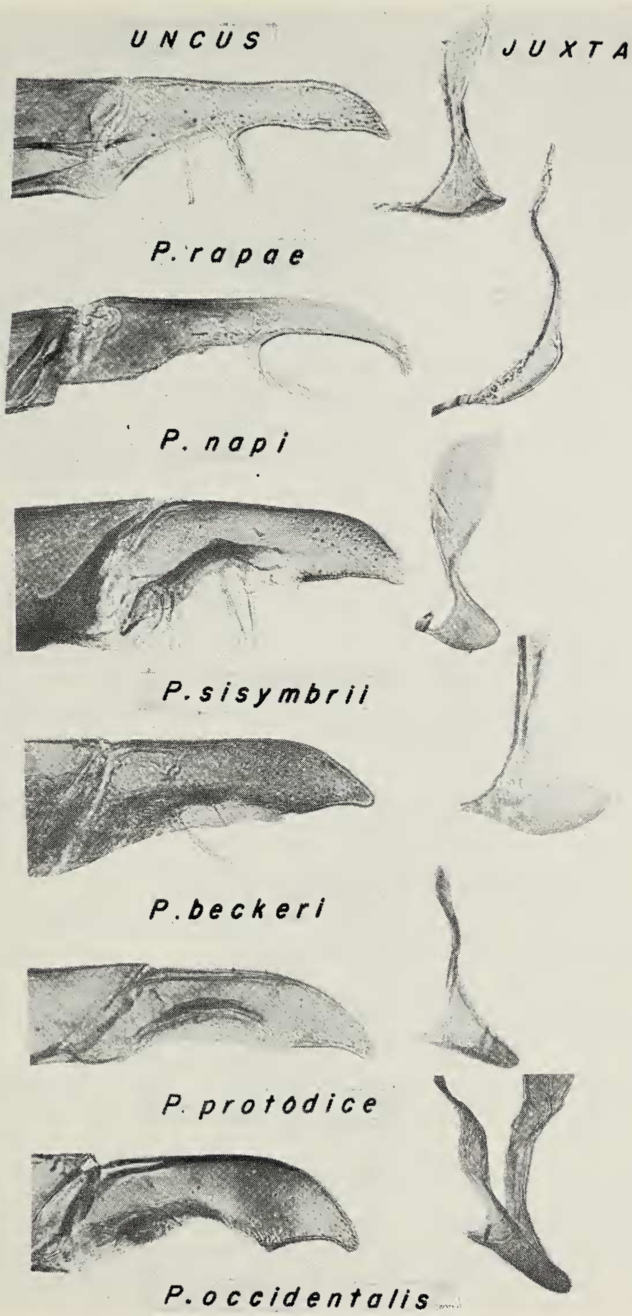

Fig. 7. Male genital structures

Table 4 Ratios between length of various genital structures and length of front wing

Saccus

Species	No.	Mean length of saccus	Mean length of front wing	Mean of ratio	S. D.	S. E.	t	Probability
rapae	9	0.51 mm	22.59 mm	2.26	0.27	0.09	1.38	0.2 +
napi	7	0.51	24.8	2.08	0.19	0.07		
sisymbrii	8	0.48	20.98	2.30	0.28	0.10	0.94	0.2 +
beckeri	8	0.51	23.7	2.17	0.24	0.09		
protodice	53	0.62	22.96	2.68	0.31	0.04	12.2	0.001
occidentalis	30	0.81	23.54	3.45	0.19	0.04		

Table 5 Ratio between length of R_3 and length of front wing

Species	No.	Mean length front wing	Mean length R_3	Ratio	S. D.	S. E.	t	Probability
P. sisymbrii	12	20.73 mm	0.89 mm	4.16	1.10	0.32	7.83	0.001
P. beckeri	12	24.82 mm	0.36 mm	1.42	0.50	0.14		
P. sisymbrii	12	20.73 mm	0.89 mm	4.16	1.10	0.32	7.86	0.001
P. protodice	34	22.79 mm	0.27 mm	1.17	1.10	0.19		

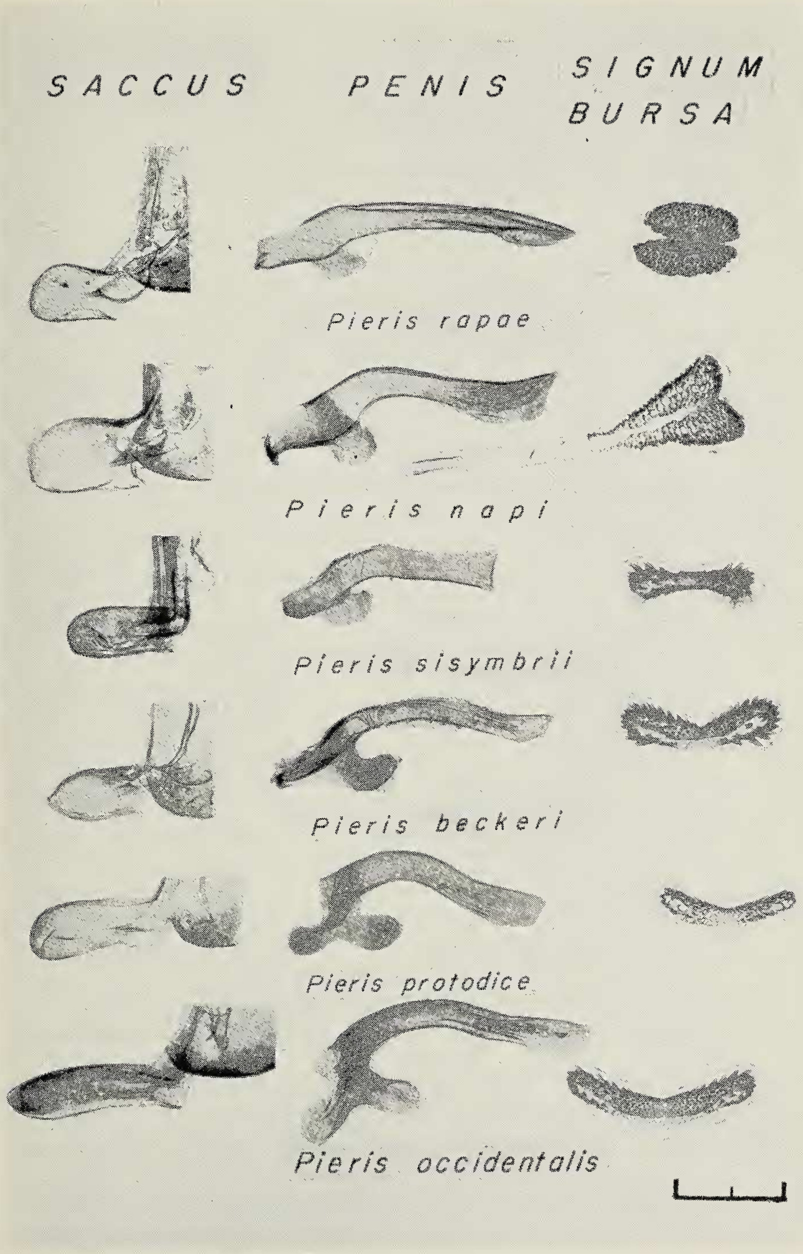

Fig. 8. Male and female genital structures

II. *P. sisymbrii* and *P. beckeri*

1. Color and pattern (fig. 1b): These two species are easily separated by the color and the pattern on the wings. *P. beckeri* has the intensified moss green color and *P. sisymbrii* has the biskra data color along the vein on the underside of the wings.

2. Venation (fig. 9, table 5): The length of R_3 between *P. sisymbrii* and *P. beckeri* differs. The ratio of fore wing length over R_3 for *P. sisymbrii* ranges from 2.34 to 5.88; for *P. beckeri* ranges from 0.95 to 2.62. The probability that the mean for the two populations could have been drawn by chance from the same population is less than 0.001 when degrees of freedom is 24 as tested by *t*.

3. Androconia (fig. 4): The scent scales of *P. sisymbrii* and *P. beckeri* are similar but unlike *P. rapae* and *P. napi*. The tiny, lobeless scales of *P. sisymbrii* are nearly parallel at the lateral border, and the roots are limited to the base. The scales of *P. beckeri* are usually irregular shaped and the roots are often occupied half the length of the scale.

4. Uncus (fig. 7, table 3): The uncus of *P. beckeri* and *P. sisymbrii* have hairy processes at the ventral side, particularly in *P. sisymbrii*. The actual length of the uncus in the two is much the same, but the ratio is different (probability 0.005; d.f. 20) due to the constant smaller size of wing length in *P. sisymbrii*.

5. Juxta (fig. 7): The juxta of *P. beckeri* and *P. sisymbrii* are similar except the apex is slightly curved upward in *P. beckeri*.

6. Saccus (fig. 8, table 4): There is little difference in shape or size with respect to this structure.

7. Penis (fig. 8): The shape of the penis in *P. sisymbrii* and *P. beckeri* is like the penis of *P. rapae* and *P. napi*. However, it is distinctly different from the penis of *P. protodice* and *P. occidentalis*. The basal protuberance of the penis of *P. beckeri* and *P. sisymbrii* is insignificant. But it rises abruptly for *P. protodice* and *P. occidentalis*.

8. Signum bursa (fig. 8, table 6): The signum bursa of *P. sisymbrii* and *P. beckeri* is stick-shaped, with large, heavily chitinized teeth. The difference between the ratios is probably caused by chance error due to the extremely small sample of *P. sisymbrii* examined, rather than to a real difference.

The samples of *P. sisymbrii* came from (*1*) Kelso Valley, Kern Co. Calif. (2), (*2*) Roads End, Tulare Co. Calif. (1), (*3*) King's Canyon, Fresno Co. Calif. (2), (*4*) Hurricane Ridge, Olympic National Park, Wash. (6), and (*5*) Whitehorse, Yukon Territory (1). The samples of *P. beckeri* came from (*1*) Long Valley, Mono Co. Calif. (1), (*2*) Mono Lake, Mono Co. Calif. (2), (*3*) Gardnerville, Douglas Co. Nevada (2), (*4*) Minden, Douglas Co. Nevada (2), (*5*) Doyle, Lassen Co., Calif. (2), and (*6*) Satus Creek, Yakima Co. Wash. (3).

Table 6 Ratios between length of various genital structures and length of front wing

Signum bursa

Species	No.	Mean length of signum bursa	Mean length of front wing	Mean of ratio	S. D.	S. E.	t	Probability
sisymbrii	2	0.75 mm	22.8 mm	3.28	0.05	0.032	3.86	0.01
beckeri	6	0.76	26.87	2.84	0.14	0.057		
protodice	35	0.74	23.32	3.18	0.195	0.03	14.4	0.001
occidentalis	25	0.96	24.0	4.03	0.25	0.05		

Table 7 Ratios between width of tornus dot and length of front wing

Species	No.	Mean length front wing	Mean width tornus dot	Ratio	S. D.	S. E.	t	Probability
P. protodice	32	22.80 mm	2.83 mm	12.38	1.33	0.23	18.48	0.001
P. occidentalis	28	22.53	1.41	6.28	1.22	0.23		

III. *P. protodice* and *P. occidentalis*

1. Color and pattern (fig. 1c, table 7): The color and pattern of these two species are many times undistinguishable, especially in comparison between the high temperature form of *P. occidentalis* and the low temperature form of *P. protodice.* The primary difference in the pattern, besides the gross one of more extensive black pigment on *P. occidentalis* males than on *P. protodice* males, is in the size and shape of the dot in the tornus area near the inner margin on the underside of the fore wings. The dot on *P. protodice* is large, square shaped and not clearly outlined, part of the dot being sometimes submerged on the uppersides. The dot of *P. occidentalis* is small, relatively clearly outlined, usually not submerged. The ratios for the width of the tornus dot range from 8.73 to 15.38 for *P. protodice;* and from 3.95 to 8.51 for *P. occidentalis.* The probability that this difference is due to chance alone is less than 0.001 for the degrees of freedom of 60 by the *t* test.

2. Venation (fig 9, 10, table 5): The R_3 veins of *P protodice* and *P. occidentalis* are quite short, sometimes being completely missing. The mean ratios of wing length over R_3 length between the two species do not show much difference. (1.17 for *P. protodice*; and 1.15 for *P. occidentalis*). However, the difference is significant between *P. protodice* and *P. sisymbrii* in comparing the ratios despite the similarities in their wing color and pattern. The mean ratio for *P. sismybrii* is 4.16; and for *P. protodice* it is 1.17. That the difference as indicated might be due to chance is less than 1/1000 for the degrees of freedom of 46 by the *t* test.

In *P. protodice,* the intersection of R_2, R_s+M_1 (point "A") is usually above point "B" where M_2 joins that vein; however, point "A" is usually on or below point "B" in *P. occidentalis.* The distance between point "A" and point "B" is measured and plotted on the diagram (fig. 11) against the ratio obtained from the width of the tornus dot to the length of the fore wing. When point "A" is above point "B" the measurement is given a "+" sign. When point "A" is below point "B" the measurement is given a "—" sign. The diagram (fig. 11) shows that *P. protodice* and *P. occidentalis* are well separated by these two characters. Few exceptional dots on the diagram as the measurements between point "A" and point "B" having a "—" sign for *P. protodice* or vice versa for *P. occidentalis* are from the specimens collected at the overlap zone of these two species.

The width of tornus dot and the location of R_2 in respect of M_2 are two pirmary characters used in the investigation to separate the two species.

3. Androconia: Thirty-five specimens of male *P. protodice* and twenty-eight specimens of male *P. occidentalis* from ten different localities were checked, but no androconial scales have ever been found. Similar results were also observed by Bernardi (1947).

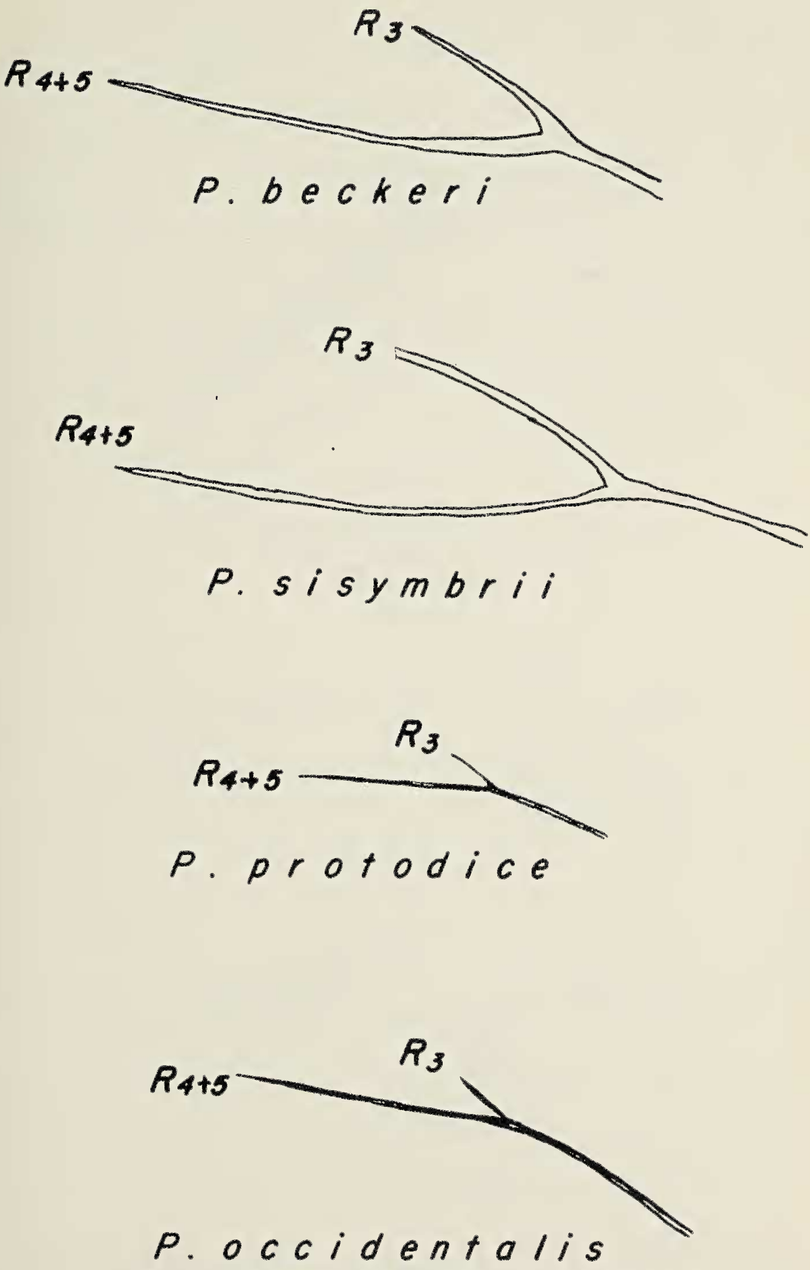

Fig. 9. Venations showing the differences in the length of R_3

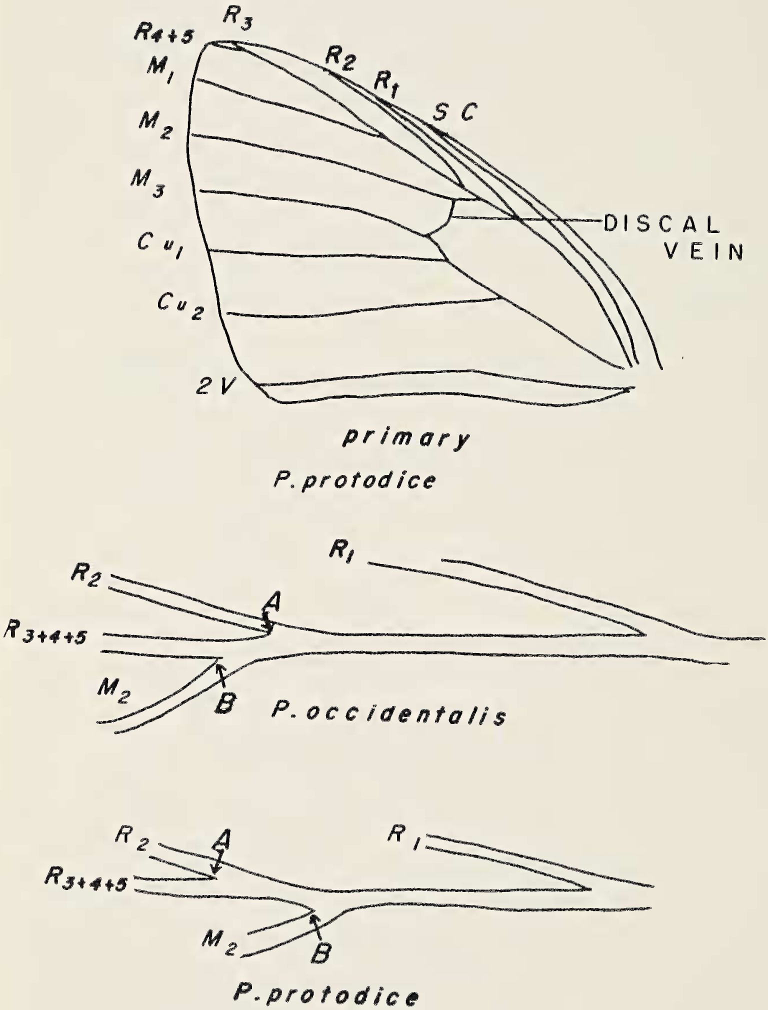

Fig. 10. Venations showing the left fore wing and the differences in the location of point "A" in respect to point "B" between *P. protodice* and *P. occidentalis*

4. Male genitalia: (1) Uncus and juxta (fig. 7, table 3): No difference is found between *P. protodice* and *P. occidentalis* in respect to these two characters.

(2) Saccus (fig. 8, 12, table 4): The length of saccus is statistically different between *P. protodice* and *P. occidentalis*. The mean ratio (length of fore wing over length of saccus) for *P. protodice* is 2.68; for *P. occidentalis* it is 3.45. The probability is less than 0.001 that the difference may be due to chance when the degrees of freedom is 83 as shown by the *t* test.

The scatter diagram (fig. 12), which shows each individual ratio of these two species against its own wing length, shows that overlap is present in this character despite the difference in mean ratio. This may be due to the variation in wing size caused by geographical and climatical difference. In cooler areas, the butterfly is usually smaller and in warmer areas, larger. If some specimens of *P. occidentalis* are from the extreme southern locations and some specimens of *P. protodice* from the extreme north, the gap in ratio between these two populations is reduced, and overlap occurs. This point is demonstrated by fig. 14. If the specimens of *P. protodice* and *P. occidentalis* are from the same locality and collected at the same time, these two species are usually separable.

(3) Penis (fig. 8): The penis of *P. protodice* and *P. occidentalis* is strongly curved at the basal part and this difference makes these two species easily distinguishable from other species in the genus *Pieris*. The penis size in P. *protodice* is smaller than in *P. occidentalis*.

5. Signum bursa (fig. 8, table 6): The signum bursa of *P. protodice* and *P. occidentalis* is similar to *P. sisymbrii*, but with smaller teeth and less chitinization. The length of signum bursa in *P. protodice* is shorter than the one in *P. occidentalis*. The mean ratio (length of fore wing over length of signum bursa) for *P. protodice* is 3.18 and for *P. occidentalis* is 4.03. The probability that this difference is due to chance alone is less than 0.001 when the degrees of freedom is 60 by the *t* test. The scatter diagram (fig. 13) for individual ratios shows that there is no overlap present between these two species with respect to this character.

The mean ratio of each population at a given locality for *P. protodice* and *P. occidentalis* is also plotted on a diagram (fig. 14) which shows that the ratios of the specimens from northern localities such as Alaska are larger than the ratios of the specimens from southern localities. The differences in ratios for the specimens from various southern localities are not significant. This is porbably due to the closeness in distance between the localities and to similarities in environment. This diagram illustrates however that the ratio is not everywhere unchanged. The ratio gradually increases in the more northern

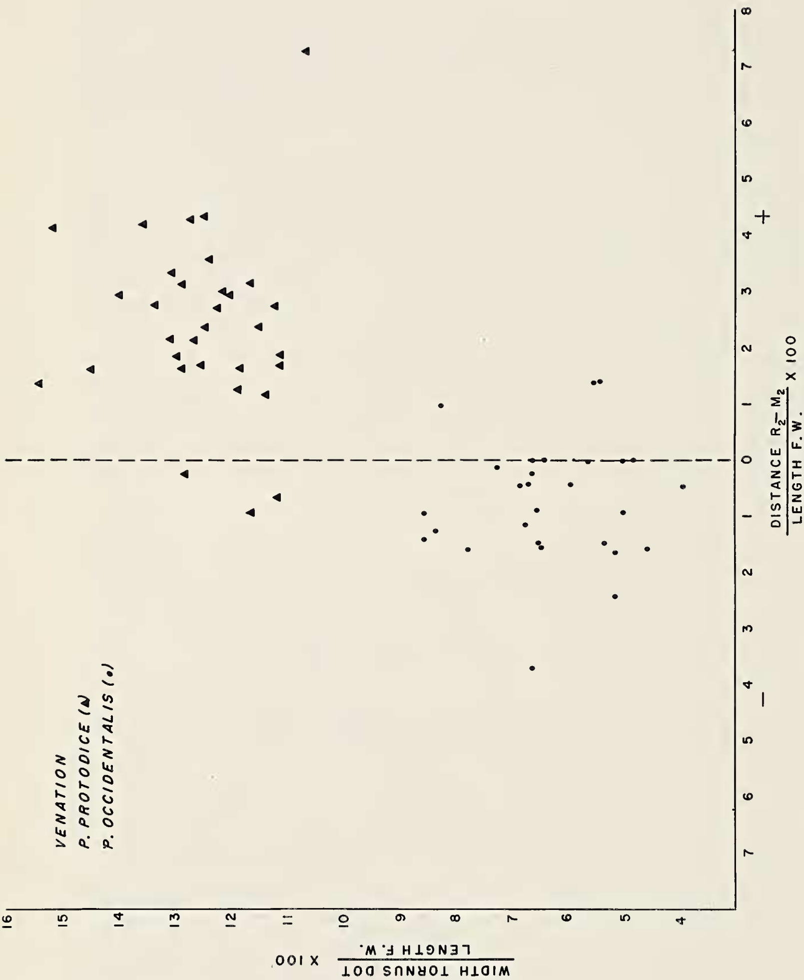

Fig. 11. The measurements between point "A" and point "B" (see fig. 10) plotted against the ratios obtained from the width of the tornus dot to the length of the fore wing

Fig. 12. Scatter diagram showing the individual ratio of saccus length to the length of fore wing plotted against the individual saccus length in *P. protodice* and *P. occidentalis*

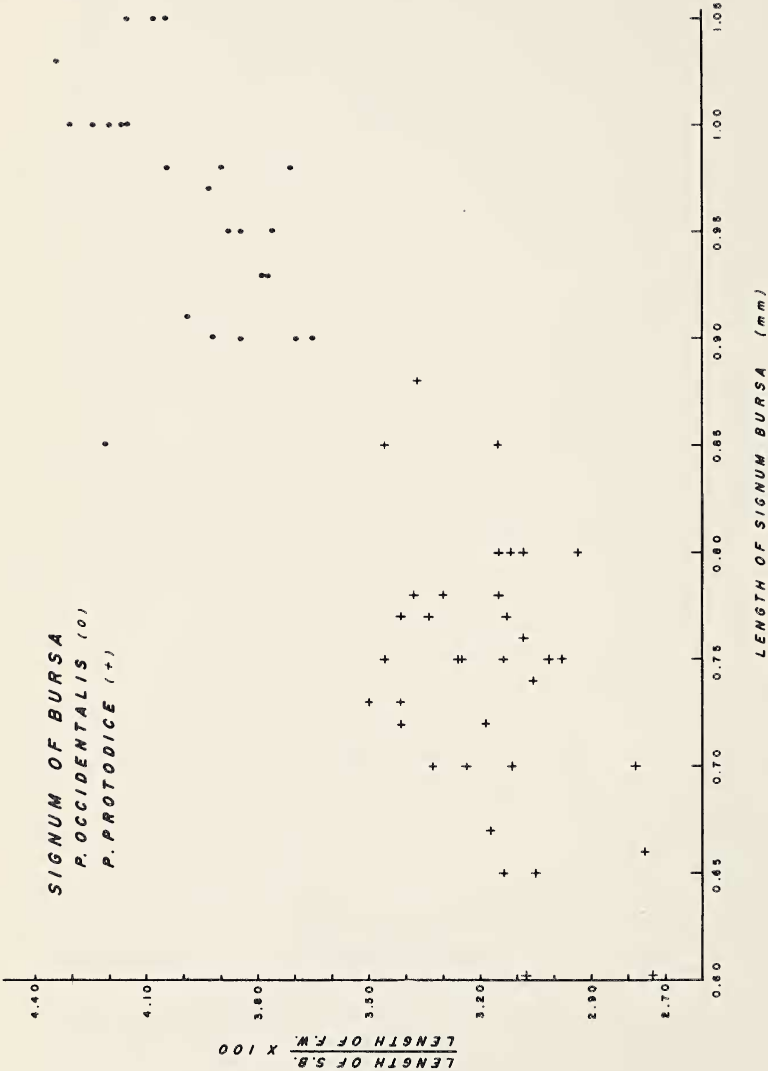

Fig. 13. Scatter diagram showing the individual ratio of the length of signum bursa to the length of fore wing plotted against the individual length of signum bursa in *P. protodice* and *P. occidentalis*

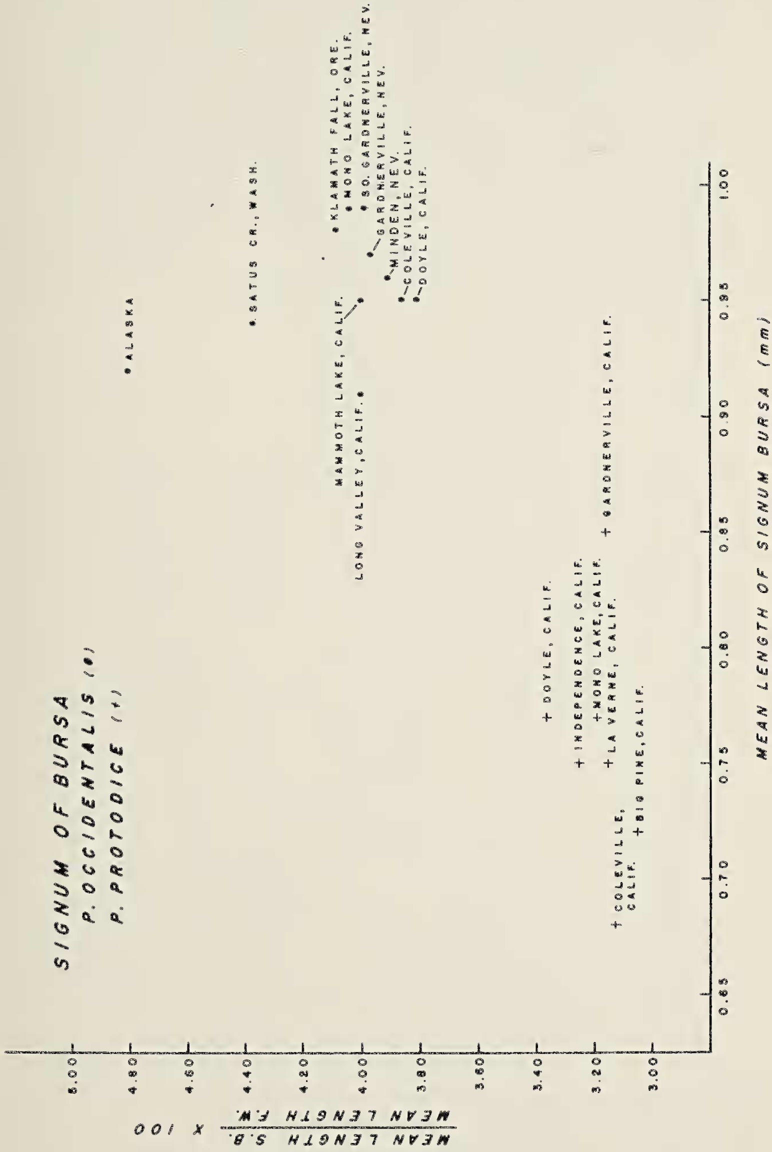

Fig. 14 Scatter diagram showing the mean ratio of the length of signum bursa to the length of fore wing in each locality plotted against the mean length of signum bursa

localities. The ratios of two species may overlap at different localities though they do not do so at the same location.

The samples of *P. protodice* came from (*1*) Laguna Canyon, Orange Co. Calif. (11), (*2*) Moorpark, Ventura Co. Calif. (4), (*3*) Independence, Inyo Co. Calif. (2), (*4*) Big Pine, Inyo Co. Calif. (2), (*5*) Mammoth Lake, Mono Co. Calif. (1), (*6*) Mono Lake, Mono Co. Calif. (4), (*7*) Coleville, Mono Co. Calif. (5), (*8*) Gardnerville Douglas Co. Nevada (1), (*9*) Minden, Douglas Co. Nevada (1), and (*10*) Doyle, Lassen Co. Calif. (4). The samples of *P. occidentalis* came from (*1*) Long Valley, Mono Co. Calif. (1), (*2*) Mammoth Creek, Mono Co. Calif. (2), (*3*) Mono Lake, Mono Co. Calif. (2), (*4*) Coleville, Mono Co. Calif. (1), (*5*) Gardnerville, Douglas Co. Nevada (11), (*6*) Doyle, Lassen Co. Calif. (3), (*7*) Klamath Falls, Klamath Co. Oregon (4), (*8*) Satus Creek, Yakima Co. Washington (2), (*9*) British Columbia (1), and (*10*) Alaska (1).

GEOGRAPHICAL DISTRIBUTION OF *P. PROTODICE* AND *P. OCCIDENTALIS*

The geographical distribution of all species in the genus *Pieris* in North America except *P. protodice* and *P. occidentalis* is thoroughly investigated by Hovanitz (1962) (fig. 15). The data here presented are mostly gained by extensive search throughout the range of *P. protodice* and *P. occidentalis* in West Coast conducted by William Hovanitz in the summer of 1962. *P. protodice* is most abundant in the south, from the southern tip of Baja California northwards, gradually reducing its population and being replaced by *P. occidentalis.* The northern border of *P. protodice* is still unclear. At present this species is only in California and Nevada southwards. There is no record in Oregon and northward. *P. occidentalis* extends from central Alaska along the Rocky Mountains and the Cascade Mountains southwards into the Sierra Nevada Mountains (and the Rocky Mountains). The most southern locality known from our data at present in the Pacific coast area is Long Valley, Mono Co., Calif. It is very likely this species may be found at more southern places on high Sierra Nevada Mountains.

P. occidentalis and *P. protodice* overlap from Long Valley, Mono Co. Calif. northward to Doyle, Lassen Co., Calif. Further investigation may extend the overlap zone more northward even to Washington.

The habitats of these two species are very similar; both like sunny, open, grassland. *P. protodice* prefers warmer temperatures and semidesert conditions. It feeds on *Brassica nigra, Caulanthus* sp., *Lepidium densiflorum, Sisymbrium altissimum, Cleome lutea,* and *Thelypodium lasiophyllum.* *P. occidentalis* prefers relatively cool temperatures. It is usally found at places relatively moist, as near a lake, ditch or farm land. The food plants of *P. occidentalis* are nearly the same as for

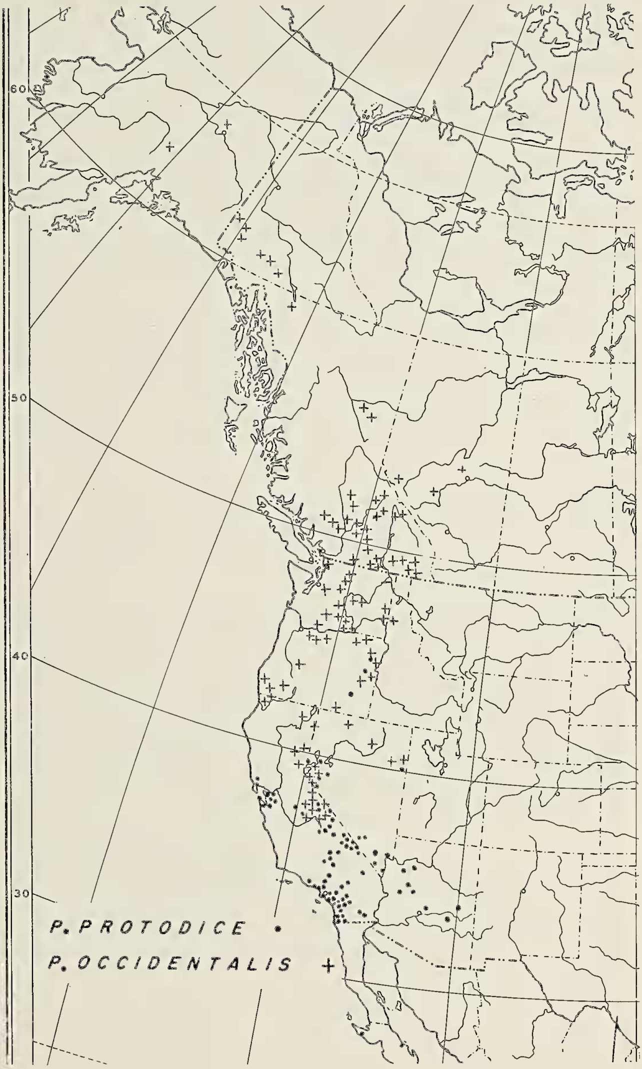

Fig. 15. Map showing the distribution of *P. protodice* and *P. occidentalis* in western North America

P. protodice except that *Cleome lutea* has not yet been recorded.

P. protodice and *P. occidentalis* are found flying together in the overlap zone. The males of these two species at a particular locality are usually separable by the marking and color on the wings, in which *P. protodice* is less darkly marked than *P. occidentalis.*

DISCUSSION AND CONCLUSIONS

The present data indicates some clear structural differences between the six species of *Pieris. P. rapae* and *P. napi* are similar in many characters, such as uncus, saccus, and the color and pattern on the wings, but they also can be distinguished by their differences in pattern and the signum bursa, juxta and androconial scales. *P. sisymbrii* and *P. beckeri* are similar in their uncus, saccus, androconial scales and signum bursa, but can usually be distinguished by wing pattern and venation. *P. protodice* and *P. occidentalis* can be distinguished by wing pattern, wing venation, structural differences in the length of saccus and signum bursa only in a statistical study. These two species also differ in their geographical distribution pattern, but overlap greatly in a sympatric area.

P. sisymbrii differs in many characters from *P. protodice,* such as wing venation, genital structures and others. The most significant difference is the presence of androconial scales, which are absent in *P. protodice* and *P. occidentalis.*

The results serve to show that *P. protodice* and *P. occidentalis* are sympatric species, an uncertainty which led Hovanitz (1962) to combine their geographical distributions into one map. The other species of the genus *Pieris* in Western North America are *P. rapae, P. napi. P. sisymbrii,* and *P. beckeri.* It is always possible that even these may be found at some time in the future to be subdivided into cryptic species by some characters not yet discovered.

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